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EXAMINATION REPORT

(PCT Rule 72.2)

From the INTERNATIONAL BUREAU

ROCHE DIAGNOSTIC Patentabteilung D-68298 Mannheim ALLEMAGNE

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Date of mailing (day/month/year)

30 January 2002 (30.01.02)

Applicant's or agent's file reference 5181/OA/WO-Im

International application No.

PCT/EP00/00602

IMPORTANT NOTIFICATION

International filing date (day/month/year) 27 January 2000 (27.01.00)

Applicant

ROCHE DIAGNOSTICS GMBH et al

1. Transmittal of the translation to the applicant.

The International Bureau transmits herewith a copy of the English translation made by the International Bureau of the international preliminary examination report established by the International Preliminary Examining Authority.

2. Transmittal of the copy of the translation to the elected Offices.

The International Bureau notifies the applicant that copies of that translation have been transmitted to the following elected Offices requiring such translation:

AU,CA,CN,JP,KR,NZ, US)

The following elected Offices, having waived the requirement for such a transmittal at this time, will receive copies of that translation from the International Bureau only upon their request:

EP,HU,IL,MX,NO,PL,RU,ZA

3. Reminder regarding translation into (one of) the official language(s) of the elected Office(s).

The applicant is reminded that, where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report.

It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned (Rule 74.1). See Volume II of the PCT Applicant's Guide for further details.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

Authorized officer

El Mostafa MOUSSAKO

Telephone No. (41-22) 338.83.38

4627265

Facsimile No. (41-22) 740.14.35

PATENT COOPERATION TREA

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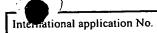
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(AY 2 0 2002N TERNATIONAL PRELIMINARY EXAMINATION REPORT

TECH CENTER 1600/2900

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference 5181/OA/WO-Im | FOR FURTHER ACTION SeeNotificationofTransmittalofInternational Preliminary Examination Report (Form PCT/IPEA/416) | | | | |
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| International application No. | International filing date (day/ | month/year) Priority date (day/month/year | ·) | | |
| PCT/EP00/00602 | 27 January 2000 (27 | .01.00) 29 January 1999 (29 | 0.01.99) | | |
| International Patent Classification (IPC) or G01N 33/68 | national classification and IPC | | | | |
| Applicant | ROCHE DIAGNOSTIC | CS GMBH | | | |
| and is transmitted to the applicant | according to Article 36. | d by this International Preliminary Examining | Authority | | |
| 2. This REPORT consists of a total o | f 5 heets, includi | ng this cover sheet. | • | | |
| amended and are the basis f 70.16 and Section 607 of th | nied by ANNEXES, i.e., sheets of or this report and/or sheets contate Administrative Instructions undustrial of sheets. | of the description, claims and/or drawings whi ining rectifications made before this Author der the PCT). | ch have been rity (see Rule | | |
| This report contains indications rel | lating to the following items: | | | | |
| Basis of the report | Basis of the report | | | | |
| II Priority | | | | | |
| III Non-establishment | t of opinion with regard to novel | y, inventive step and industrial applicability | | | |
| Lack of unity of invention | | | | | |
| V Reasoned statemen | V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement | | | | |
| VI Certain documents | s cited | | | | |
| VII Certain defects in | the international application | | | | |
| VII 🖂 | ns on the international applicatio | n | | | |
| VIII Certain observatio | appround | | | | |
| Date of submission of the demand | Date | of completion of this report | | | |
| 25 May 2000 (25.0 | 5.00) | 10 May 2001 (10.05.2001) | | | |
| Name and mailing address of the IPEA/EF | Autho | rized officer | | | |
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| 1. With regard to the elements of the international application:* | | | | | | |
| [| | the inter | national application as originally filed | | | |
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| | the i | nternationse element the land the land the land or 55.3 | o the language, all the elements marked above were available and application was filed, unless otherwise indicated under the swere available or furnished to this Authority in the followage of a translation furnished for the purposes of internguage of publication of the international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguage of the translation furnished for the purposes of international application (underguag | r this item. owing language national search (under l er Rule 48.3(b)). nternational prelimina | Rule 23.1(b)). | which is: |
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| | . Rep | The and this report 170.17). | mendments have resulted in the cancellation of: the description, pages the claims, Nos the drawings, sheets/fig eport has been established as if (some of) the amendment of the disclosure as filed, as indicated in the Supplemental sheets which have been furnished to the receiving Officer as "originally filed" and are not annexed to this ment sheet containing such amendments must be referred | Box (Rule 70.2(c)).** e in response to an inv report since they do | itation under A not contain a | rticle 14 are referred to mendments (Rule 70.16 |
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| V. | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement | | | | |
|----|---|--------|--------|-----|--|
| 1. | Statement | | | | |
| | Novelty (N) | Claims | 5 - 19 | YES | |

| Novelty (N) | Claims | 5 - 19 | YES |
|-------------------------------|--------|--------|-------|
| | Claims | 1 - 4 | NO |
| Inventive step (IS) | Claims | | YES |
| | Claims | 1 - 19 | NO NO |
| Industrial applicability (IA) | Claims | 1 - 19 | YES |
| | Claims | | NO |

2. Citations and explanations

Reference is made to the following documents:

D1: WO-A-93/24531

D2: WO-A-89/12069

D3: Clin. Endocrinol. (1997) 47 287-296

1. Novelty (PCT Article 33(2)):

The subject matter of Claim 1 is anticipated by D1 1.1 (D1: Claim 9). D1 describes in detail the production of the monoclonal antibody 1C7 (Example 1; pages 7-8). The method described, including the cloning, can be reproduced by a person skilled in the art. Accordingly, a person skilled in the art is also able to produce a second antibody directed against BNP(1-76), which then also enables a sandwich assay to be carried out (D1: page 9, paragraph 1; Claims 7-9). Although the antibodies of the present application produced by peptides (application: page 20, Table 1) do not detect native pro-BNP, this does not mean that the antibodies produced as per D1 (Example 1) likewise do not detect the native pro-BNP since other peptides are used in D1 (BNP(1-21), BNP(22-46), BNP(47-64)).

Therefore the novelty of Claims 2 to 4 is also anticipated by D1.

- 1.2 Claim 5 is novel since none of the available documents discloses a method which uses two antibodies that detect different antigenic determinants of N-terminal proBNP and have a detection limit of less than lfmol/ml patient blood (= 1 pmol/l).
- 1.3 Claim 6 is novel since none of the available documents discloses a BNP-based detection method which permits differentiation between healthy patients and those suffering cardiac insufficiency of NYHA Classes I IV. Accordingly dependent Claim 7 is likewise novel; the same applies to independent use Claim 8.
- 1.4 Claim 9 is novel since none of the available documents discloses recombinant N-terminal proBNP.

 Accordingly the use of recombinant N-terminal proBNP (Claims 10 and 11), specific antibodies (Claims 12 to 16), their production (Claims 18 and 19) and the associated cell lines (Claim 17) are also novel.
- 2. Inventive step (PCT Article 33(3)):
- 2.1 The single additional technical feature in Claim 5 concerns the result to be attained with the invention, namely a low N-terminal proBNP detection limit (< 1 fmol/ml). Since the method of detecting N-terminal proBNP in a sample (Claim 1) is not novel, the claimed detection limit appears to be attainable by normal experimentation with specific

antibodies (PCT Article 33(3)).

2.2 Claim 6 does not appear to be inventive: D3, which is considered the closest prior art, specifies that the N-terminal proBNP (NT-proBNP) can be used as a cardiac insufficiency marker. The NTproBNP level in NYHA Class 1 patients is higher than in healthy patients and increases as the cardiac insufficiency increases, the D3 assay permitting differentiation between healthy patients and NYHA Class I - IV patients (D3: page 287, column 2, paragraphs 2, 3; page 291, Figure 3). The method for determining NT-proBNP which is used in D3 is a radio immunoassay that uses an antiserum produced to act against human proBNP (1-13) (D3: page 288, column 2, paragraph 1). Consequently D3 differs from the subject matter of Claim 6 by the detection method. Whilst two antibodies detecting different antigenic determinants on N-terminal proBNP are used according to Claim 6, the D3 method uses only one antibody in a competitive test format (D3: page 288, column 2, paragraph 1).

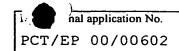
The object achieved in Claim 6 is that of preparing a sensitive assay which only requires short incubation periods (see application, page 4, paragraph 3).

A person skilled in the art is familiar with sandwich assays with two antigen-specific antibodies (e.g. D1: Claim 9; page 8, last line - page 9, paragraph 1), and with their advantages such as, for example, the shorter incubation periods (D1: page 9, paragraph 1). Accordingly the teaching of D3, that is, the use of NT-proBNP as marker for

distinguishing between healthy plasma and NYHA Class I - IV plasma in conjunction with a sandwich ELISA appears to suggest the subject matter of Claim 6. Therefore **Claims 7 and 8** likewise appear to be non-inventive.

2.3 Claim 9 does not appear to comply with PCT Article 33(3). The significance of N-terminal proBNP as diagnostic indicator or predictor for cardiac insufficiency has been known for a long time (e.g. D1: page 3, lines 2-6) and the use of antibodies against the N-terminal proBNP peptide in immunoassays in particular is described in the prior art (e.g. D1: page 3, line 7 - page 5, line 11). D1, which is considered the closest prior art, describes the production (D1: page 6, paragraph 3) of proBNP (1-76) by chemical synthesis, which is a long-known standard method of producing peptides. Moreover, D1 describes the use of N-terminal proBNP(1-76) for producing antibodies (D1: Claim 15). However, D1 does not describe the production and use of recombinant BNP(1-76).

The object achieved in Claim 9 is consequently that of devising an alternative method for producing a proBNP(1-76) peptide. D2 discloses the cDNA sequence of human BNP. Figure 5 (D2: page 16, lines 24-27) discloses the DNA sequence of human BNP (coding region of the plasmid phBNP-1) and the associated peptide sequence. Example 5 (page 41) of D2 describes the cloning of human BNP. Moreover, D2 describes the production of BNP in recombinant expression systems (D2: page 20, line 18 - page 26, line 25).



The significance and use of N-terminal proBNP is described in D1.

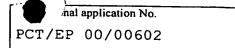
Furthermore, the production of a recombinant protein on the basis of a known cDNA sequence is a standard technique for a person skilled in the art.

In conclusion, the sequence disclosed in D2 in combination with the significance and use described in D1 appears to render the subject matter of **Claim** 9 obvious.

Since the production of antibodies with peptides and proteins is likewise a familiar technique for a person skilled in the art and is also described, for example, in D1 (pages 7-8), Claims 12, 14-16, 18 (D1: page 9, paragraph 2) and 19 (D1: page 7, paragraph 2 - page 8, paragraph 3) likewise appear not to comply with PCT Article 33(3).

Claim 13 concerns antibodies against the 10-66 range of amino acids of N-terminal proBNP. The choice of this range is intended to ensure that the analyte can also be detected when the N or C-terminal amino acids have already been digested by proteases. D1 can be cited against this since it describes the monoclonal antibody 1C7 which specifically binds to the BNP(47-64) sequence. Moreover, the D1 method can also produce an antibody which is directed against the BNP(22-46) peptide (D1: page 7, Example 1, paragraph 1). Therefore Claim 13 appears to be lacking an inherent inventive concept.

The same applies to Claims 15 to 17, which concern specific deposited antibodies and the producing cell



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| VII. Certain defects in the international app | plication |
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The following defects in the form or contents of the international application have been noted:

Contrary to the requirements of PCT Rule 5.1(a)(ii), the description did not cite D2 and it did not briefly outline the relevant prior art contained therein.